



Pollution from Personal Actions, Activities, and Behaviors: Pharmaceuticals and Personal Care Products in the Environment



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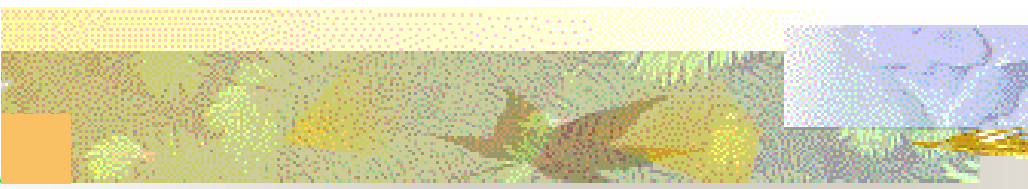


Summary of some research being conducted on PPCPs by EPA-Las Vegas and collaborators

Presentation made at *The 10th Symposium on Handling of Environmental and Biological Samples in Chromatography*, 1-4 April 2001, Mainz/Wiesbaden, Germany

Session on "Pharmaceuticals in the Environment"
[organized and chaired by Dr. Thomas Ternes]

<http://www.uni-mainz.de/~eswe/symposium.htm>



U.S. EPA Notice

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U.S. EPA, Office of Research and Development
National Exposure Research Laboratory
Environmental Sciences Division
Las Vegas, Nevada





Primary Goals of the U.S. EPA's *Office of Research and Development*

- **Identification of potential (future) environmental concerns:** forward thinking, planning, and research.
- **Proactive vs. Reactive — Pollution prevention vs. remediation/restoration:** Identify and foster investigation of “hidden” or potential environmental issues/concerns before they become critical ecological or human health problems.
- **Ruling-in/ruling-out vs. Uninformed rules:** Provide bases for informed decisions. Ensure that science leads eventual decisions for guidance or to regulate/not regulate.
- **Foster interdisciplinary research & collaboration:** Catalyze research by academe, private sector, government.



Pharmaceuticals and Personal Care Products (PPCPs)

Fact: Certain PPCPs occur in the environment
(esp. the aquatic environment)

Origins: Domestic sewage, hospitals, CAFOs

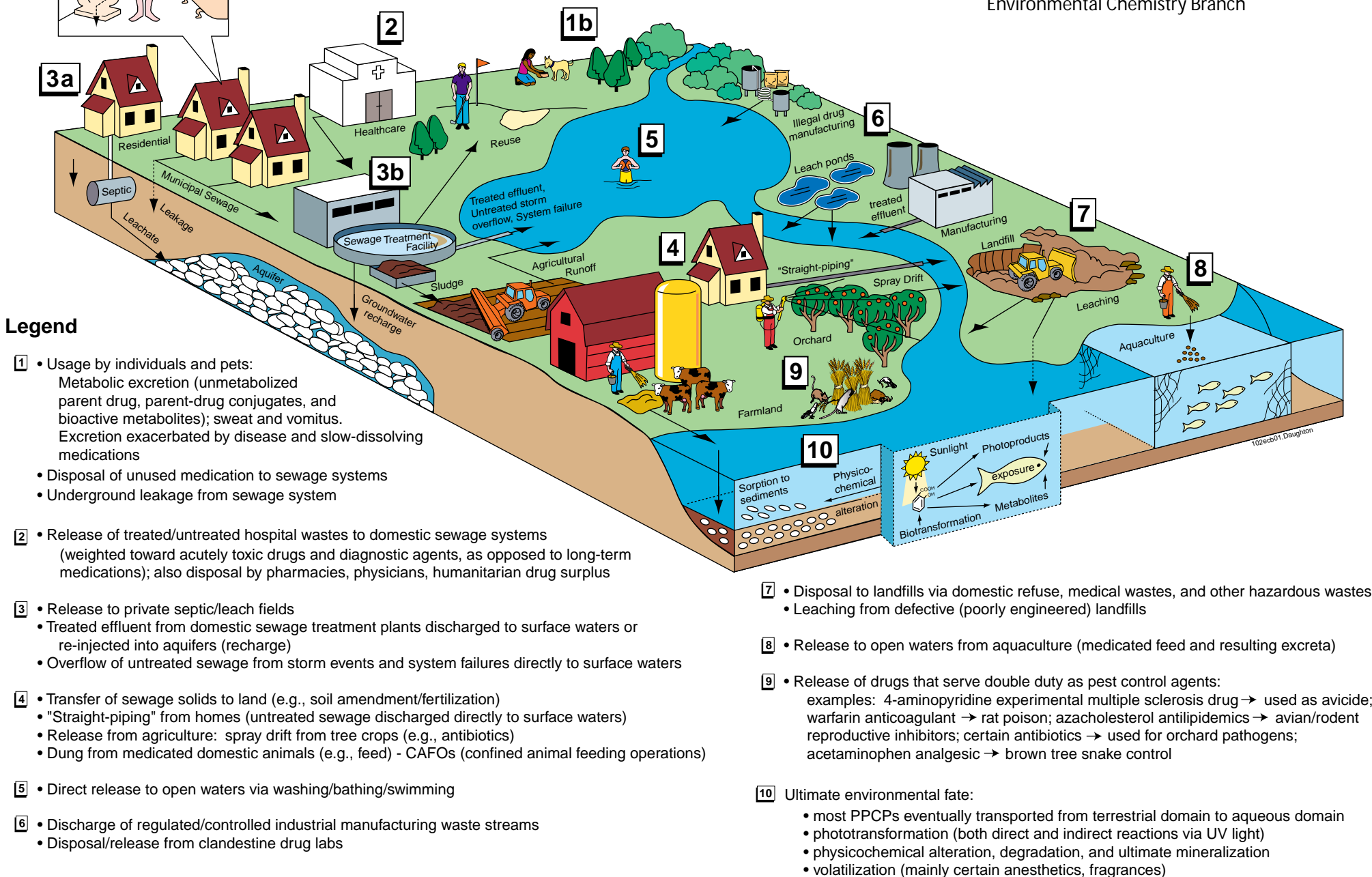
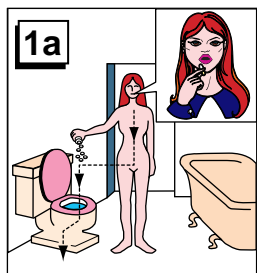
Issue: Fate and effects are poorly understood
Numerous questions...

Origins and Fate of PPCPs† in the Environment

†Pharmaceuticals and Personal Care Products



U.S. Environmental Protection Agency
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National Exposure Research Laboratory
Environmental Sciences Division
Environmental Chemistry Branch





Major Tasks for Science Community

- ▶ Determine which therapeutic or consumer-use classes of PPCPs have an environmental presence and what their **trends** are.
- ▶ For each PPCP class known to be present in the environment at significant individual or combined concentrations, rule-in or rule-out possible deleterious environmental effects.
- ▶ Task will involve simultaneous work from both exposure and effects scientists working in parallel and in sequence.



Importance of acquiring TRENDS data

- ▶ Acquisition of trends data for a suite of PPCPs (representatives from each of numerous significant classes), shown to recur amongst municipal STWs across the country, is of key importance.
- ▶ Trends data would prove critical if the current concentrations are below any known effects levels.
- ▶ A frequent criticism of the concern with this issue is that the limited data on environmental occurrence points to concentrations well below *therapeutic levels*. One of several limitations to this argument is that there are no data whatsoever that show whether what are currently trace concentrations are serving as a foundation on which more significant concentrations are slowly building — using trends data to predict well in advance the onset of effects levels.
- ▶ If environmental concentrations begin to trend upward, this could trigger renewed or expanded examinations or trigger more rigorous pollution prevention efforts. Both short-lived and long-lived PPCPs should be selected for trends monitoring.

– *continued*–




... because ...

Finally, there are potential, intangible advantages in being proactive versus reactive ...

► the **Precautionary Principle** – the principle of precautionary action that redistributes the burden of proof because the science required for truly and fully assessing risks lags far behind the requisite supporting science:

e.g., see: <http://www.pmac.net/precaut.htm>

– concluded –



Majority of PPCP classes have no environmental survey data

- ▶ Environmental survey data have yet to be reported for many classes (and class members) of PPCPs.
- ▶ While the literature is silent regarding these PPCPs, is this because of an absence of data or a failure to report “data of absence”?
- ▶ Many of these unreported drugs are among the most widely prescribed in the U.S.

continued –

PPCPs with no environmental survey data

(bolded names among top 200 most prescribed in U.S.: <http://www.rxlist.com/top200a.htm>)

therapeutic class	example generic names (many drugs cross over into multiple classes)	example Brand names
adrenergic receptor inhibitors (anti-BPH agents)	terazosin , doxazosin , finasteride	Hytrin, Cardura, Proscar/Propecia
amyotrophic lateral sclerosis	riluzole	Rilutek
analgesics (non-NSAIDs and narcotic)	tramadol , propoxyphene , oxycodone , hydrocodone	Darvon, Ultram, Tylox
anorexiant (diet drugs)	fenfluramine, orlistat	Pondimin, Xenical
Antiarrhythmics	disopyramide, flecainide, amiodarone, sotalol	Norpace
Anticoagulants	warfarin	Coumadin
antidepressants	esp. SSRIs (sertraline , paroxetine , fluoxetine , fluvoxamine), tricyclics (desipramine), MAOIs (phenelzine), misc.	Zoloft, Paxil, Prozac, Luvox, Wellbutrin (bupropion), Serzone (nefazadone), Effexor (venlafaxine)
antidiabetic agents	insulin sensitizers, antihyperglycemic (e.g., sulfonylureas)	Rezulin (troglitazone), Glucophage (metformin), Glucotrol (glipizide), DiaBeta (glyburide)
antihistamines (H-1 blockers)	fexofenadine , loratadine , cetirizine , terfenadine	Allegra, Claritin, Zyrtec, Seldane

PPCPs with no environmental survey data

histamine (H-2) blockers	famotidine, ranitidine, nizatidine	Pepcid, Zantac, Axid
Decongestants	Ephedrines	
anti-infectives	many special disease classes (amebicides, anti-fungals, malarials, tuberculosis, leprosy, viral) & chemical classes	Diflucan (fluconazole)
antimetabolites	methotrexate	Rheumatrex
antipsychotics, CNS agents	alprazolam, zolpidem, clonazepam, risperidone, temazepam thioridazine, rifluoperazine	Xanax, Ambien, Klonopin, Risperdal, Restoril
calcium-channel blockers	diltiazem, nifedipine, amlodipine, verapamil	Cardizem, Procardia, Norvasc
digitalis analogs	digoxin , digitoxin	Lanoxin
diuretics	thiazide (hydrochlorothiazide , chlorthalidone); loop (furosemide, bumetanide); potassium-sparing (spironolactone, triamterene)	Lasix (furosemide) Dyazide (hydrochlorothiazide , triamterene)

PPCPs with no environmental survey data

dopamine agonists	anti-Parkinsonian agents (e.g., pramipexole, ropinirole)	Mirapex, Requip
expectorants	guaifenesin	Entex
gastrointestinal agents (ulcer drugs)	omeprazole, lansoprazole, cimetidine	Prilosec, Prevacid, Tagamet
HIV drugs	protease inhibitors, anti-retrovirals (nucleoside analogs/reverse transcriptase inhibitors)	Crixivan (indinavir), Retrovir (zidovudine)
hormonally active agents androgens anti-acne agents adrenocortico steroids inhalable steroids estrogen antagonists	fluoxymesterone isotretinoin, tretinoin prednisone, triamcinolone fluticasone tamoxifen	Accutane, Retin-A Flovent Nolvadex
muscle relaxants	cyclobenzaprine	Flexeril
osteoporosis agents	alendronate sodium	Fosamax
prostaglandin agonists	latanoprost	Xalatan

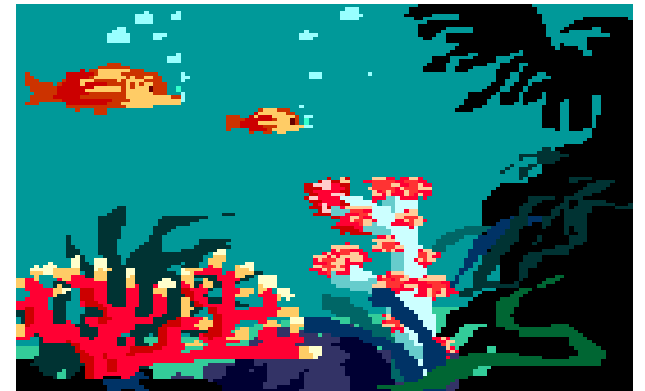


PPCPs with no environmental survey data


psychostimulants (amphetamine-like)	methylphenidate, dextroamphetamine	Ritalin
sexual function agents	sildenafil citrate	Viagra
street drugs (illicit, illegal, recreational)	many: e.g., see listing at: “Streetdrug.org” (http://www.mninter.net/%7epublish/index2.htm)	
vasodilators (esp. angiotensin converting enzyme [ACE] inhibitors)	lisinopril, enalapril, quinapril, benazepril losartan, fosinopril, ramipril	Zestril, Vasotec, Accupril, Lotensin Cozaar, Monopril
newly approved, upcoming, and investigational drugs	Ongoing: see listing at: “Lexi-Comp.org” (http://www.lexi.com/new_drugs.htm)	
“chemosensitizers”, efflux pump inhibitors (EPIs)	verapamil (and others from diverse classes; e.g., http://www.microcide.com/ICAAC99Posters/icaac99_posters.html)	

Aquatic organisms — captive to continual, life-cycle chemical exposures

► **Aquatic Exposure is Key:** Any chemical introduced via sewage to the aquatic realm can lead to continual, multigenerational exposure for aquatic organisms.



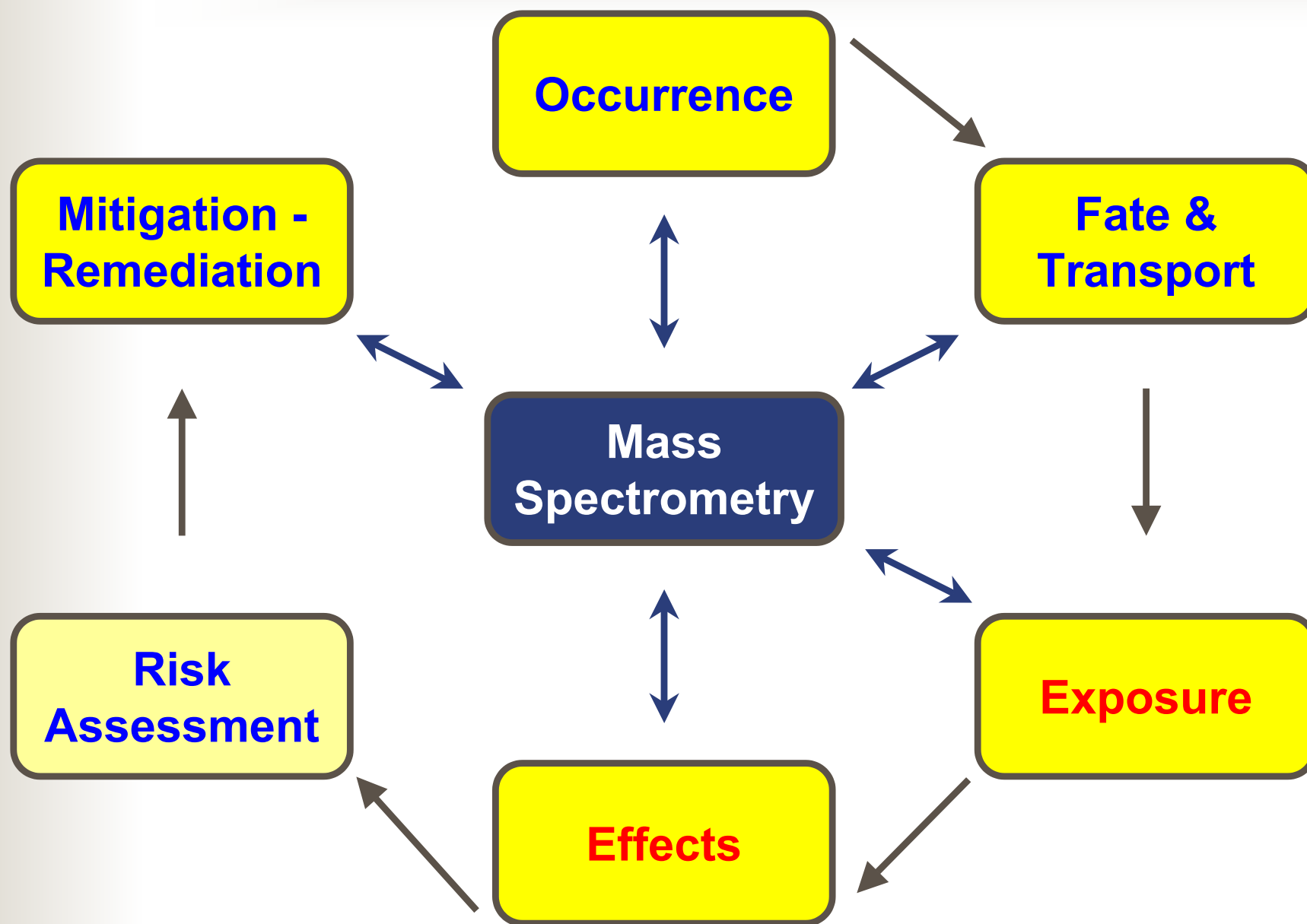
► **Re-evaluation of “Persistence”:** Chemicals continually infused to the aquatic environment essentially become “persistent” pollutants even if their half-lives are short — their supply is continually replenished (analogous to a bacterial chemostat).



Current EPA Projects at Las Vegas focusing on sampling and enrichment

- **Enrichment of Personal Care Products – synthetic musks**
- **Enrichment of polar water-soluble PPCPs via “POCIS” –
in collaboration with USGS (CERC-Columbia)**
- **Concentration and detection of polar PPCPs via SPE and
μLC-ES/ITMS**

Mass Spectrometry and the Risk Paradigm





Solid Phase Extraction & Enrichment of Synthetic Musks

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Solid Phase Extraction (SPE)

- About 27 years ago, Junk et al., Musty, and Nickless pioneered the use of SPE for direct extraction/enrichment of environmental samples.
- Early studies focused on Amberlite XAD-2&4.
- In 1979, Rees and Au used methanol/XAD-2 slurry for good recoveries of pesticides.
- 2001, EPA scientists and others using poly(methylmethacrylate) mixed 1:1 with polystyrene divinylbenzene.
- Commercially available as ***Varian Absolut NEXUS***

On-site SPE Enrichment of Synthetic Musks

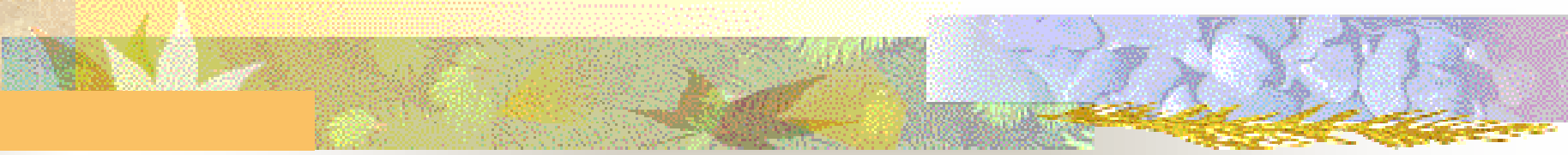
using 1:1 poly(methylmethacrylate):polystyrene divinylbenzene

- Enrichment factor approaches 1,000,000 : 1
- No pretreatment/activation of resin
- Integrates temporal variations in analyte concentrations
- Only dissolved-phase analytes are extracted (particulates excluded)
- Resultant sample is highly compact, easily stored, and transported
- Minimizes biohazards in STW samples. Simplified extraction
- Sample extracts are analyzed by conventional GC/MS.
- High signal/noise enables unknown to be authenticated with NIST mass spectral library



Table 1. % Spike Recovery data from 60-L Sample (n=3)

Analytes	Tap-water	STP Effluent	MDL (ng/L)
Musk xylene	102	97	0.02
Musk ketone	98	95	0.20
Musk ambrette	101	96	0.30
Musk moskene	96	92	0.03
Musk tibetene	98	95	0.02
Versalide	99	96	0.02
Galaxolide	99	97	0.02
Phantolide	97	91	0.02
Cashmeran	99	94	0.02
Celestolide	98	96	0.02
Traseolide	95	90	0.02
Tonalide	107	94	0.02
4-Amino musk xylene	87	80	0.30
2-Amino musk xylene	89	82	0.25
Amino musk ketone	90	92	0.25



Current Status in the Development of an Integrative Sampler for Polar Organic Chemicals in Water

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Columbia, MO**



Polar Organic Chemical Integrative Sampler (POCIS)

- ♦ **Many polar organic contaminants (pesticides, pharmaceuticals, etc.) have been linked to acute toxicity and chronic sublethal effects of various organisms.**
- ♦ **Most standard methods do not accurately sample a wide range of polar contaminants and very few provide time-weighted average (TWA) concentrations.**
- ♦ **The POCIS was developed to sequester and concentrate waterborne polar organic chemicals thus providing TWA concentrations and organism exposure estimates.**

Polar Organic Chemical Integrative Sampler

The POCIS consists of a microporous hydrophilic polymeric membrane containing an admixture of solid phase sequestering media (20% carbonaceous Ambersorb 1500 dispersed on S-X3 BioBeads and 80% modified polystyrene divinylbenzene resin)



The POCIS is currently undergoing laboratory calibration and field validation.

An U.S. Government patent application describing the POCIS device is under review.

The POCIS has been successfully deployed in a constructed wetland system and is currently in use at a confined animal feeding operation (CAFO) site.

Future deployments include continued study of the environmental impact of CAFO operations and WWTP effluents in a collaboration with the U.S. Environmental Protection Agency and the UK Environmental Agency.

Representative Contaminants Tentatively Identified in Wastewater and Constructed Wetland Complex

POCIS data

Atrazine

Hydroxyatrazine

Desethyl-Deisopropyl Atrazine

Alkyl Phenols (Nonyl Phenol)

Caffeine

Ibuprofen

Propoxur

Phenelzine

Mephenytoin

Ethotoin

Nicotinic Acid

17 α -Ethinylestradiol

17 β -Estradiol-3-sulfate

Oxytetracycline

Sulfadiazine

Sulfamerazine

Sulfadimethoxine

Oxindole

4-Hydroxyindole

5-Phenylhydantoin

Tri-2-butoxyethyl phosphate

Vitamin A

Using laboratory calibration data, atrazine was quantified at 0.84 ppb which is in general agreement with a previously reported value¹ of 1.16 ppb from a nearby site.

NASQAN report:

<http://water.usgs.gov/nasqan/data/provdata/hermann.html>

Development and Application of a Solid Phase Extraction and Micro-Liquid Chromatography-Electrospray/Ion trap Mass Spectrometry Method for Detecting Pharmaceuticals in Natural Waters



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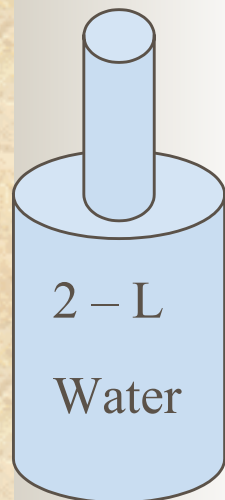




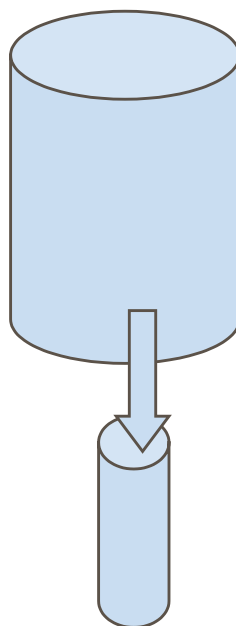
Objective

- To develop an analytical method that can detect non-volatile, polar, water-soluble pharmaceuticals in natural waters at levels that could be environmentally significant (at concentrations less than parts per billion, ppb).

300 μ L of HCl (12 N)



❖ Accuprep 7000™ manifold and
48-mm (or 92-mm) nu*phase
SPE C₁₈ discs (CPI
International)

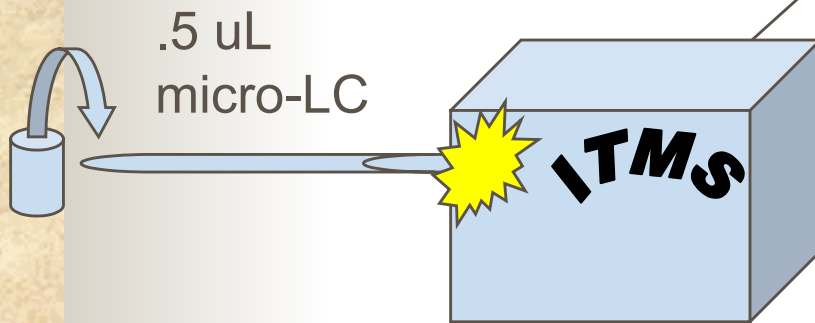


❖ Extract with four 20-mL (or 60-mL) volumes
of 99% methanol/1% acetic acid

❖ Evaporate to 0.3 mL using
TurboVap® II (Zymark Corporation,
Hopkinton, MA, USA)

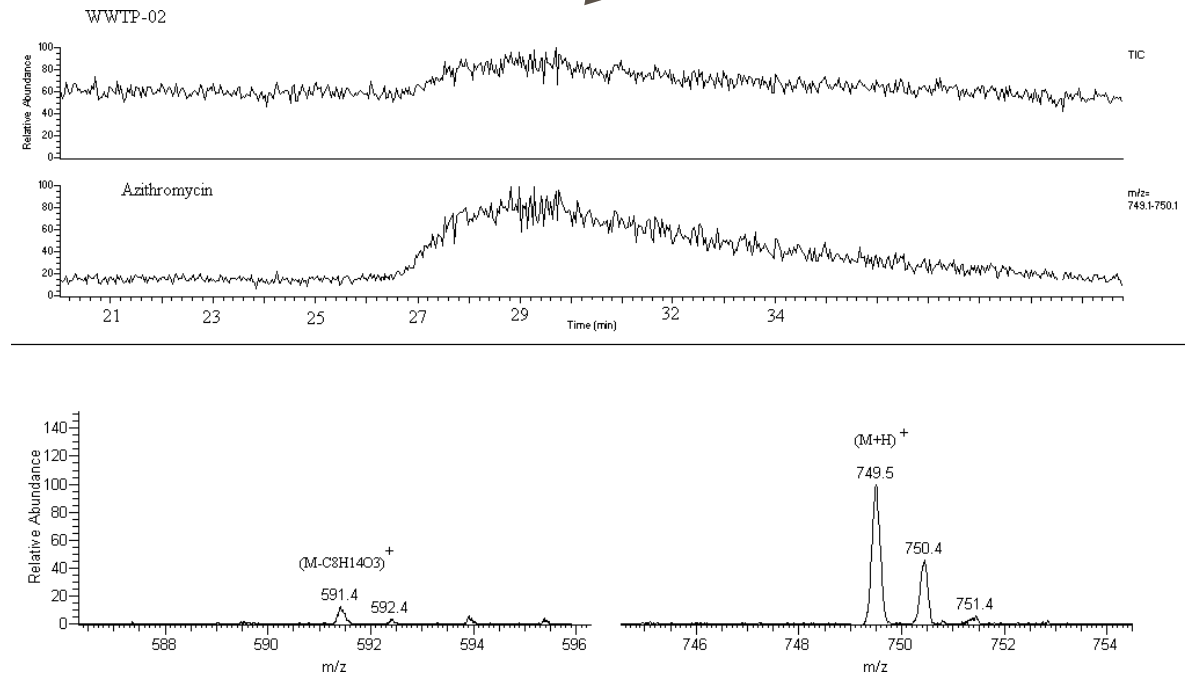
Final extract to LC –
ITMS for analysis





Finnigan LCQ™, configured with an electrospray (ES) ion source:

- positive ion mode
- ES needle: 4.8 kV
- 180 to 800 amu



Ion Chromatogram and Mass Spectrum of Azithromycin



Targeted Analytes

Compound (# on RxList – 1999 http://www.rxlist.com/top200.htm)	Molecular Weight Amu	Molecular Formula
Fluoxetine (10)	309.33	$C_{17}H_{18}F_3NO$
Omeprazole (4)	345.42	$C_{17}H_{19}N_3O_3S$
Azithromycin (16)	749.0	$C_{38}H_{72}N_2O_{12}$
Levothyroxine Na^+ (2)	798.86	$C_{15}H_{10}I_4NNaO_4$



LODs* of Targeted Analytes (*limits of detection)

Analyte	LOD ng
Azithromycin	4
Levothyroxine	1
Omeprazole	1
Fluoxetine	6

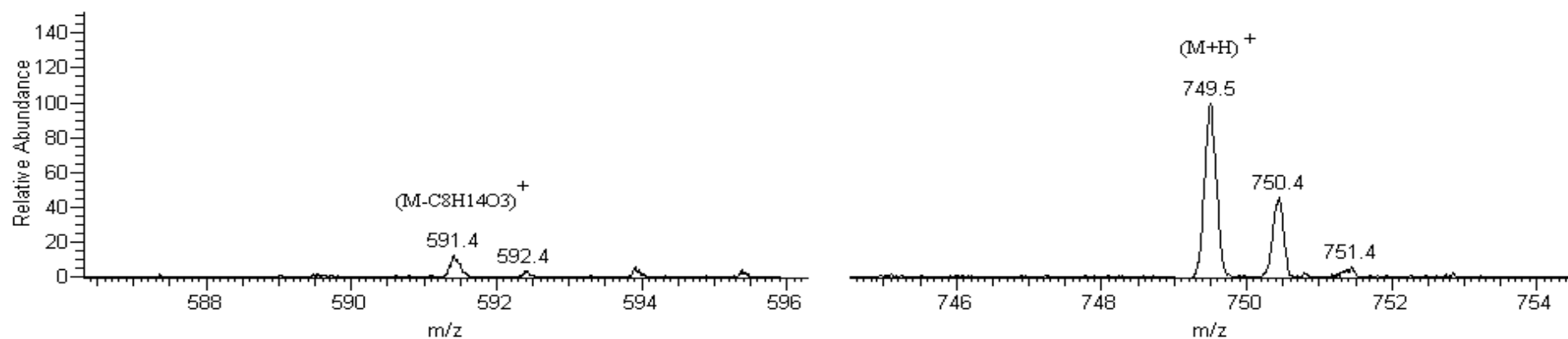
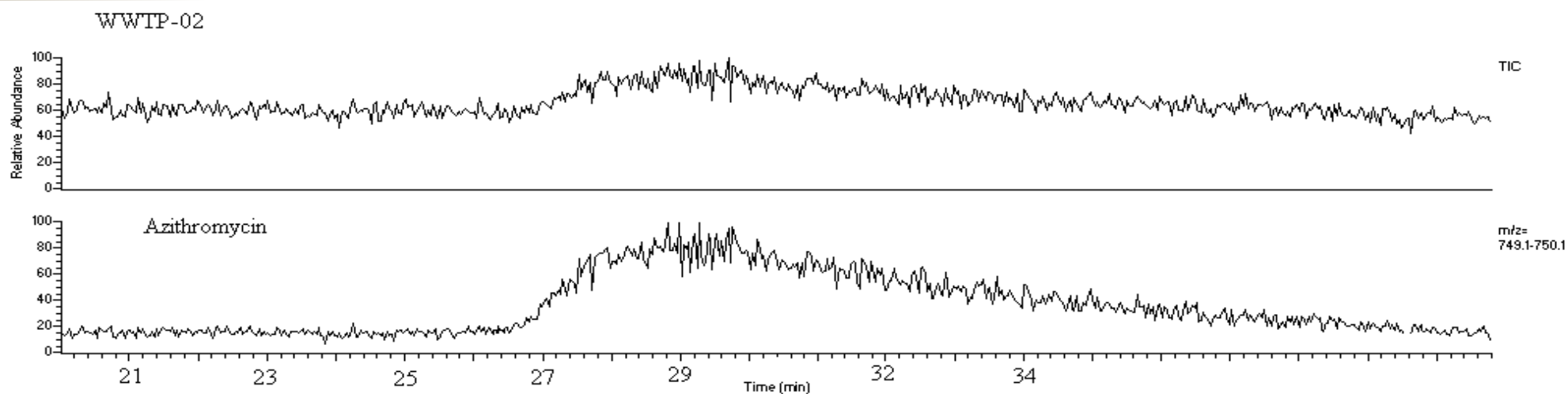


Targeted Analytes

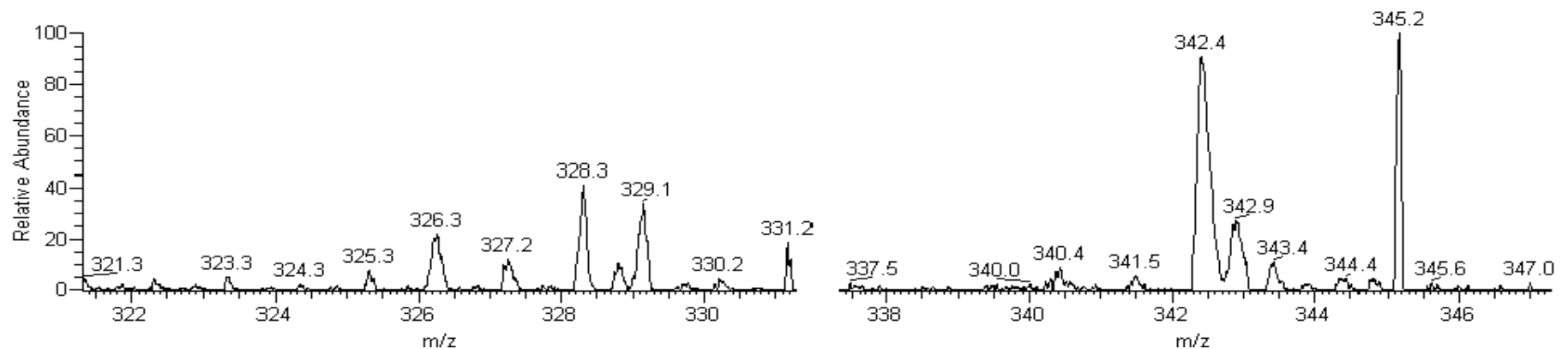
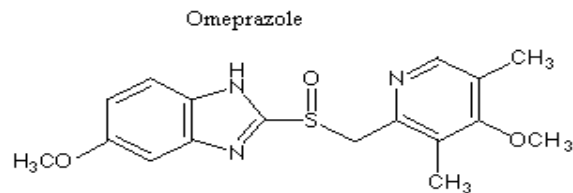
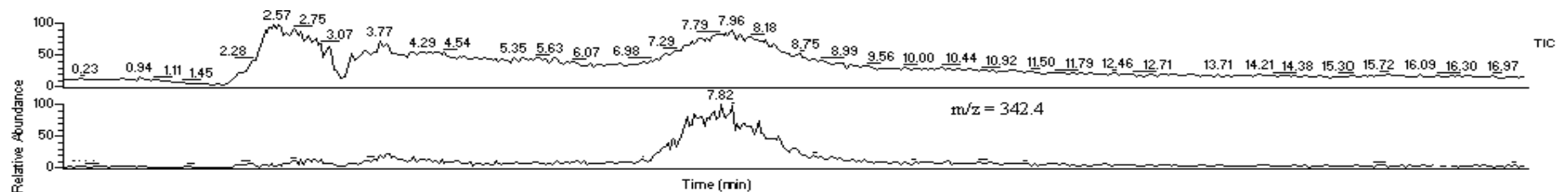
% Recoveries from Spiked Waters using SPE C18 Discs

Compound	% Recovery (% RSD)
Azithromycin	67 (27)
Levothyroxine	114 (20)
Fluoxetine	88 (9)
Omeprazole	26 (19)

Ion Chromatogram and Mass Spectrum of Azithromycin



Ion Chromatogram and Mass Spectrum of Omeprazole





Summary of Analytes (both targeted and non-targeted) found in WWTP effluent samples

Levothyroxine (2° and 3°)

Azithromycin (2°)

Omeprazole (2°)

Fluoxetine (3°)

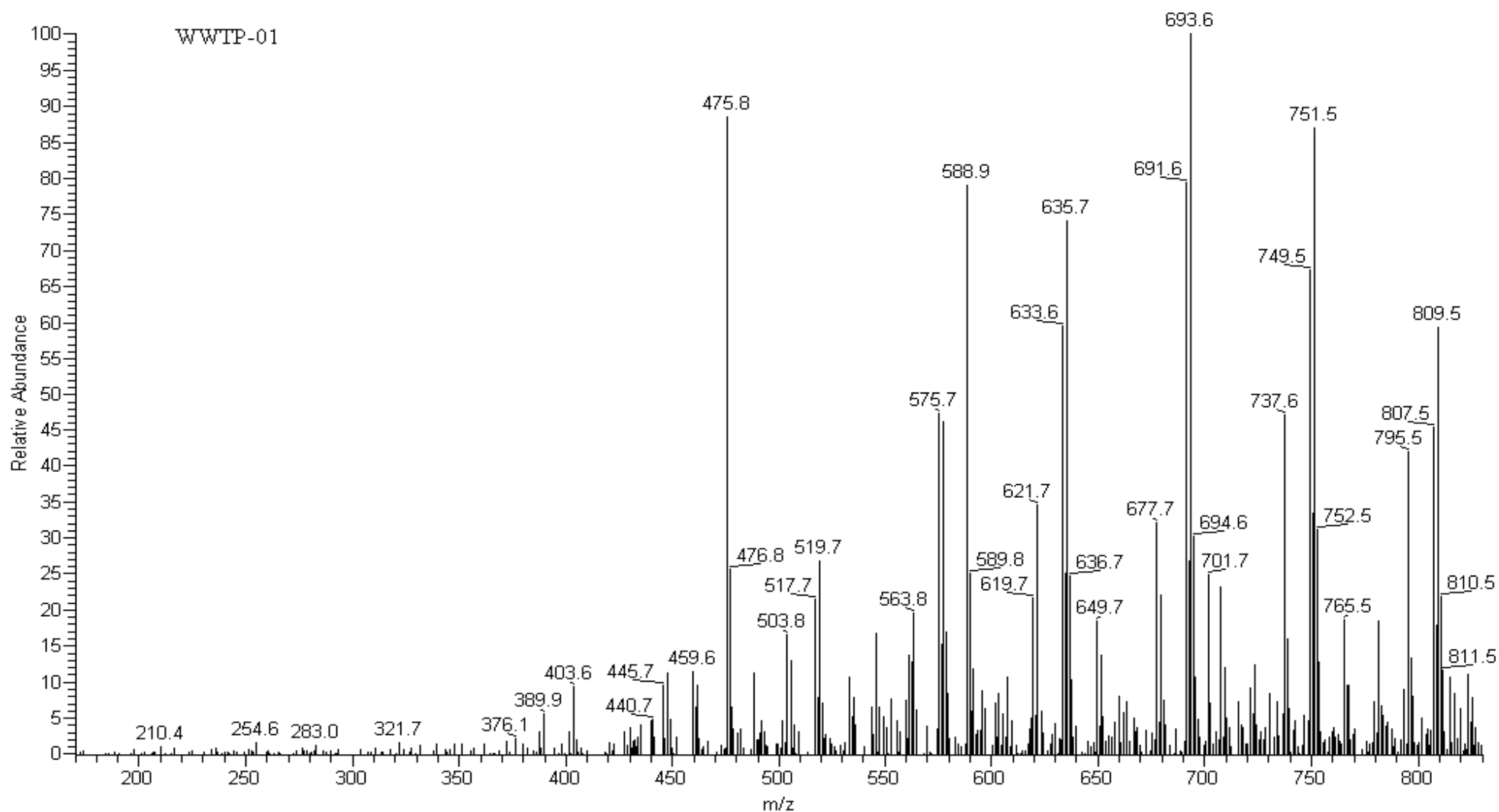
Polyoxypropylene glycolates (PPGs)

- PPGs are widely used in the cosmetics industry as thickeners and in shampoo formulations

Polyoxyethylene glycolates (PEGs)

- PEGs are widely used as additives in the food industry

Tentatively Identified Compounds (a mixture of PPGs and PEGs)





Summary of SPE – μ LC-ES/ITMS Method

- Analytical method is sensitive. LODs \approx 1 ng for most of the targeted analytes in the LCQ ZOOMTM mode.
- Method uses “green chemistry”: < 5 mL of mobile phase per day; < 40 mLs solvent per extraction.
- Certain of the targeted pharmaceuticals are present, at low levels, in wastewater effluents (azithromycin, levothyroxine, omeprazole, and fluoxetine).
- Many other untargeted, non-volatile and polar compounds are possibly present in wastewater effluent (e.g., PPGs and PEGs).

Near-term actions to minimize introduction of drugs to environment or their potential effects

- **Screening for EPI Potential:** Develop new aquatic testing procedures (esp. cellular based); evaluate possible impacts of potent, new-generation efflux pump inhibitors (EPIs).
- **Environmental “Friendliness”:** Factor environmental proclivity into PPCP design/marketing “green” PPCPs: maximize biodegradability/photolability to innocuous end products, minimize therapeutic dose (“calibrated dosing”), single-enantiomers.
- **Drug Prescribing & Use:** Better inform physicians (and public) to environmental consequences of over-prescribing medications — minimize misuse/overuse. Engage medical community to develop guidelines. Identify pathogens prior to prescribing antibiotics (“imprudent use”).

continued -



Near-term actions (cont'd)

- ▶ **Internet Dispensing:** Educate/encourage the pharmacy community to understand environmental consequences of over-dispensing (and dispensing without a prescription) to minimize unneeded drug use and attendant disposal [see: www.fda.gov/oc/buyonline].
- ▶ **Individualization of Therapy:** Encourage tailoring drug dosages to the individual (esp. long-term maintenance drugs) on basis of body weight, age, sex, health status, and known individual drug sensitivities — individualization of therapy. Identify lowest effective dosages (“calibrated dosing”).



▶ *continued –*



Near-term actions (cont'd)

- ▶ **Develop alternative delivery mechanisms:** Reduce dosages with (1) new routes for existing and new drugs (e.g., inhalable, dermal), (2) new formulations, and (3) new mechanisms for delivery of drugs to the target (e.g., antibody-linked drugs).
- ▶ **Expand exploration of non-chemical alternatives to traditional medications:** Reducing/eliminating drug dosages by use of placebos [e.g., see refs at: “Medicinal Mimicry: Sometimes, placebos work—but how?” D. Christensen, *Science News* 3 Feb. 2001, 159(5), 74-75,78; <http://www.sciencenews.org/20010203/bob1ref.asp>]

continued -



Near-term actions (cont'd)

- ▶ **Consider reducing package sizes of PPCPs:** Some PPCPs are perhaps more prone to being disposed because they are prescribed or purchased in quantities too great to be used before expiration or because they tend to expire more rapidly.
- ▶ **Encourage patient compliance:** Noncompliance by the patient can result in prescribed courses of a particular drug to accumulate, leading to the expired/unused dosages to be disposed in the domestic sewage system.
- ▶ **Lessen drug abuse** (consumption of more frequent/higher doses than prescribed or use of illicit drugs) to reduce excretion.
- ▶ **Reduce need for disposal** by reducing prescribed/purchased quantities too great to be used before expiration, or increasing shelf life.

continued -

Near-term actions (cont'd)

- ▶ **Proper Disposal:** Better inform pharmacy industry to provide proper disposal instructions to end-user for unused/expired drugs. Better guidance for disposition of non-controlled substances by disposal companies.
- ▶ **Importance of Individuals' Actions:** Educate public on (i) how their individual actions each contributes to burden of PPCPs in the environment, (ii) how PPCPs can possibly affect aquatic biota, and (iii) the advantages accrued by conscientious/responsible disposal and usage of PPCPs.
- ▶ **Use of Drugs as Environmental Markers of Sewage:** Capitalize on occurrence of certain, more easily degraded PPCPs to serve as conservative markers/tracers of discharge (early warning) of raw (or insufficiently treated) sewage.



continued -



Near-term actions (cont'd)

- ▶ **Expand ecotox considerations for new drug classes:** Of potential high importance is the possible need to more closely evaluate potential ecological effects from the pending introduction of new drug classes. A currently evolving example is that of the **angiogenesis inhibitors**. This broad therapeutic class has a number of synthetics – both legacy drugs, such as thalidomide, and many new ones. These compounds have **profound teratogenic potential** (thalidomide being a well-known example), but little is known with respect to aquatic organisms. [overview at: <http://www.students.stedwards.edu/~ehouran/intro.html/>]

– *concluded* –



Inter-Connectedness of Humans and the Environment

- ▶ Occurrence of PPCPs in the environment mirrors the **intimate, inseparable, and immediate** connection between the actions and activities of individuals and their environment.
- ▶ PPCPs owe their origins in the environment to their worldwide, universal, frequent, and highly dispersed but cumulative usage by multitudes of individuals.

Questions?

feel free to contact:

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